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Role of polypeptide growth factors in normal and abnormal growth

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There are three general conditions in which cell growth occurs in animals. The first is during embryonic growth and subsequent development into an adult organism. Second, there is the cellular proliferation in response to an injury such as wound repair and, of special interest here, in compensatory growth. Finally, there is the rapid growth associated with the neoplastic transformation of cells and resulting tumor growth. In this short review we will present several examples of the best characterized factors which have been associated with one or more of these situations. As with most biologically active factors, it is impossible to make clear cut-cut dividing lines as to function and several factors can be implicated several places. For example, nerve growth factor may play a role both in normal neuronal development and wound repair. Also, growth factors active in neoplastic growth may be involved in embryonic development. Both these cases will be mentioned in detail later.

Throughout this review, growth will be defined so as to include both an increase in the number of cells, hyperplasia, and the enlargement and extension of individual cells, hypertrophy. There are instances, however, where a distinction must be made. There has been an explosive proliferation in research findings concerning growth factors in the last 10 years, partially made possible by the commercial availability of several of the best characterized factors. Numerous literature makes it necessary to omit certain details here and, thus, no attempt will be made to detail the most recent theories on how growth factors transmit the mitogenic message once they interact with the target cells. For example, several recent reviews discussed in detail how this may occur as well as structural similarities of several factors [1–3].

In brief, we will summarize the current knowledge of growth factors in relationship to the three common situations where they are known to act; normal growth, response to injury, and neoplastic growth. The participation of growth factors has been well established in these situations, although there are other factors involved such as the substratum supporting the cells [4, 5] and growth-inhibiting substances [6, 7], some of which will be mentioned where necessary.

Normal development and functions

Epidermal growth factor. Epidermal growth factor (EGF) is perhaps the best characterized and certainly the most studied of all the growth factors. EGF was identified initially in extracts of mouse submaxillary glands [8, 9]. The primary biological activity used to isolate EGF was premature eyelid opening in neonatal mice. Control newborn mice open their eyes at 12 days after birth while mice injected with 1 μg/g body weight of purified EGF open them at 8 days and 0.25 μg/g at 10 days. EGF isolated from mouse submaxillary glands is an acidic polypeptide with an isoelectric point of 4.6 and molecular weight of 6045 whose complete amino acid sequence is known [10]. EGF is usually found associated in tissue with a high molecular weight carrier protein which has been identified as an arginine esterase which releases the terminal amino acids from precursors of EGF [10]. A molecule with properties similar to mouse EGF has been isolated from human urine [11]. In addition, it has been found that mouse EGF and human urogastrone share a common amino acid sequence and biological activity [12–13]. These two peptides have a common 37 residues out of a total of 53. Human urogastrone will promote early eyelid opening in mice and purified EGF can block hydrogen chloride release from gastric mucosa.

Epidermal growth factor has been shown to stimulate DNA synthesis and cell division in the nanograms per milliliter range in numerous types of cultured cells, usually of epithelial or endothelial origin [14–16]. Since EGF is easily extractable and purified from mouse submaxillary glands [9] and is active in a wide variety of cultured cell lines, it has become a valuable reagent for cell culture and is a vital component of most chemically defined media [17, 18]. A great deal of knowledge is available concerning the mode of action of EGF in stimulating cell proliferation. The probable mode of action of EGF, as well as other growth factors, may involve endocytosis of the occupied cell surface receptors and subsequent action within the cell [19, 20]. There is also evidence that EGF becomes chemically bound to its receptor [21, 22], and it has been shown recently that antibodies to the purified receptor for EGF will mimic some of the biological effects of the EGF [23]. The final determination of its exact mode of action has yet to be determined.

The most interesting aspect of EGF for this presentation is its astoundingly wide variety of actions in vivo. As mentioned above, EGF was originally isolated using the precocious eye opening in newborn mice. In addition to this, studies in fetal lambs have indicated that EGF stimulates epithelial growth in a variety of sites, especially in upper and lower respiratory airways [24]. Similar results have been reported in rabbits [25]. In adult mice, EGF has been found to stimulate DNA synthesis

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in the cecum, colon, and rectum as soon as 4 hr after injection [26]. EGF also stimulates ornithine decarboxylase activity in neonatal mouse stomach and duodenal tissue [27] and adult rat liver DNA synthesis [28]. This stimulation of epithelial cells in a variety of sites raises the possibility that EGF may be a fetal growth factor responsible for the proliferation of this cell type during normal embryonic development.

Nerve growth factor. Extracts of mouse submaxillary gland also contain another potent growth factor, nerve growth factor (NGF). This polypeptide is isolated from the male mouse submaxillary gland as a high molecular weight complex commonly termed 7S NGF [29, 30]. As with the EGF described previously, the 7S NGF is a complex composed of an arginine esterase which converts a 22,000-dalton precursor polypeptide to a 13,000-dalton NGF [31, 32] and afterwards serves as a carrier molecule. The active low molecular weight segment of NGF has been sequenced [33, 34]. The knowledge of the sequence of NGF allows it to be placed in a subset of growth factors termed the insulin-related growth factors due to its similarity in sequence and structure to insulin [34]. Other members of this subset include the multiplication-stimulating activity (MSA) [35] and the two insulin-like growth factors, I and II, which have been sequenced recently [36]. These growth factors have a similar biological property in that they all cause a so-called pleotypic response in sensitive cells. This response is usually defined as a general increase in cellular metabolism involving DNA and protein synthesis and nutrient transport. A pleotypic response need not, however, lead to eventual cell proliferation. In fact, NGF is not directly mitogenic for its target cells.

The biological activity of NGF differs considerably from that found with EGF. These two growth factors represent the opposite ends of the spectrum of activities generally ascribed to growth factors, hyperplasia and hypertrophy. NGF is not mitogenic for cultured nerve cells but acts as a survival agent. In the embryo NGF acts to stimulate both sensory and sympathetic nerve cells [39, 40]. The NGF is mitogenic only during early embryonic development, later only a hypertrophic response is seen. In vitro, NGF is limited to a strictly survival role: In its presence, cultures of NGF thrive; without it, they quickly disintegrate and die [37–40]. Similar effects may also appear in in vivo experiments. For example, in the adult, elimination of NGF by an autoimmune state or passive immunization leads to degeneration of both sensory and sympathetic neurons [38, 41]. Thus, as far as nerve cells are concerned, NGF in adults is mainly a survival agent while in the embryo NGF has a more active mitogenic role.

Recently, another interesting possible role of NGF has been found. It was originally observed that mice whose salivary glands had been removed experienced delayed wound healing [42]. This has been expanded by the finding that the topical application of NGF to wounds on mice without salivary glands lead to an acceleration of wound closure [43]. This may partially explain the presence and high levels of NGF in submaxillary glands. This is an example of the same growth factor having a role in both normal embryonic development and response to injury.

Colony stimulating factor/macrophage growth factor. The continual proliferation and maturation of bone marrow-derived cells is one of the most important growth factor mediated processes in mature animals. This continual production is necessary to replace cells lost due to aging and involvement in certain inflammatory reactions. An important subset of blood cells we will deal with here are the granulocytes and macrophages. The production of these cells from the marrow is controlled by a group of factors called colony stimulating factor (CSF). The CSF activity is commonly assayed in vitro by determining the number of granulocyte/macrophage colonies formed in soft agar from a suspension of murine bone marrow cells.

Colony stimulating activity has been collected from a variety of sources. CSF has been found in submaxillary glands [44] as have the above-mentioned EGF and NGF. Another source is human or mouse serum [45, 46]. The most common source is media conditioned by growth of various types of cultured cells, mainly the murine fibroblast L-cell line [47, 48]. The CSF purified from this source is a 60,000- to 70,000-dalton glycoprotein with an isoelectric point of 3.7 to 4.9. Reduction of the CSF with 2-mercaptoethanol results in the formation of two subunits of about 35,000 daltons which are devoid of biological activity. A lower molecular weight factor, 23,000 daltons, with CSF activity has also been reported from mouse lung fibroblasts [49]. On gel filtration columns under nondenaturing conditions, the CSF apparently aggregates into a large molecular weight species of 190,000 daltons.

The purified CSF is active in vitro to promote colony formation from murine marrow cells at a concentration of approximately 3 ng/ml or 10^{-11} M [47, 48]. The purified CSF has been used to raise specific anti-CSF antibodies [48, 50]. These antibodies have been used to show that the in vivo halflife of injected CSF is about 1.5. The antibodies do not inhibit in vivo granulopoesis but do show measurable effects in peritoneal diffusion chambers [48, 51].

A structurally similar growth factor has been reported which is capable of stimulating the proliferation of functionally mature macrophages. This activity differs from the CSF in that the CSF stimulates the maturation of granulocytes and macrophages from common stem cells while the macrophage growth factor (MGF) is a direct mitogen for mature macrophages. A MGF from mouse lung fibroblast conditioned media has been purified extensively [52, 53]. It is a 68,000-dalton glycoprotein with an isoelectric point of 4.2. On polyacrylamide gel electrophoresis under nondenaturing condition, multiple bands are seen at progressively higher molecular weights possibly due to aggregation of the 68,000-dalton monomer.

The biological target cell of this MGF is the mature macrophage from the peritoneal exudate or a lung lavage. The dosage for stimulating proliferation of these cells is about 10^{-8} M, several orders of magnitude greater than that required for colony formation by CSF. The strikingly similar chemical properties of mouse L-cell CSF and MGF suggest that they may represent the same or very similar molecules with different effects depending on concentration [53]. The concentration of MGF would be expected to be very high at sites of inflammatory reactions leading to local expansion of the population of functionally active macrophages. Outside the inflammatory lesion, much lower levels would maintain the necessary level of production of replacement cells.

T-cell growth factor. Another factor which can stimulate the proliferation of a mature, functionally active population of cells

is the recently recognized T-cell growth factor (TCGF) or in the more current nomenclature, Interleukin-2 (IL-2). The IL-2 is one of the soluble factors which can regulate the immune response [54–56]. IL-2 activity is found in a variety of conditioned media produced by activated lymphocyte populations. The activation can be produced by a variety of substances, mitogens, alloantigens, or other foreign antigens [57–60]. The murine T-cell derived IL-2 is a protein of 30,000 daltons [60]. It is composed of two subunits with isoelectric points of 4.3 and 4.9. The rat and human IL-2 factors are of somewhat lower molecular weights, between 20,000 and 12,000 [57–59]. The isoelectric points of the rat and human IL-2 activity is in the acid range, 5.5 to 6.5.

The biological importance of the IL-2 or TCGF resides in its ability to cause expansion of clones of activated T-cells [61–63]. The factor is essential for the continued culture of T-cell blasts in culture with the maintenance of differentiated functions. The IL-2 does not stimulate lymphocyte proliferation in the absence of a previous antigenic or mitogenic signal. In this aspect, the IL-2 is similar in action to the MGF as it only stimulates a functionally mature cell population. Both these factors are important in the regulation of the inflammatory response in fully developed animals.

Injury repair

In addition to embryonic and developmental growth, a multicellular organism is also constantly required to repair various forms of damage to its integrity. This process involves both wound repair such as closure of skin after punctures or cuts and the regeneration of organ mass and function after damage caused either by mechanical means or by chemicals. In this section we will cover several examples of growth factors which may be involved in each of these processes.

Platelet-derived growth factor. The in vitro culture of animal cells requires the presence of some kind of serum for maximal growth and survival. The various factors in serum responsible for the growth-promoting activity have been a source of intense investigation. The major growth-promoting activity in serum, platelet-derived growth factor (PDGF) has been isolated and characterized by several groups. The discovery of this factor was based on the observation that cells would proliferate in culture in media containing serum but not plasma. Subsequently, it was shown that PDGF was stored in the alpha granules of blood platelets until released by the clotting process [64–67]. The PDGF has proven to be an essential component of the media for cultured cells and, in addition, has provided valuable information on the general mechanisms by which serum and plasma components both contribute to the maintenance of cell proliferation in culture.

The PDGF has been purified recently by several laboratories [68–72]. It is a basic polypeptide with an isoelectric point of 9.8 to 10.2 and about 13,000 daltons under reducing conditions. Highly purified PDGF is active in stimulating cultured cells at the 1 ng/ml level.

The in vitro activity of this factor has been a subject of intense study. The PDGF supports the growth of a variety of cell types, mainly of mesenchymal origin [64]. This includes fibroblasts mainly, but activity also includes glial cells [73] and, most importantly, primate arterial smooth muscle cells [74]. Mechanistic studies [64, 75–78] have shown that the PDGF controls some early point in the Go phase of the cell cycle and that other factors, present in both serum and plasma, are also necessary for cell growth.

The in vitro specificity of PDGF for mainly connective tissue cells suggests the role that PDGF may play in vivo. The PDGF may be involved in the repair of tissue following an injury, a classic wound hormone. The packaging of the PDGF in the granules of the platelet is also supportive of this suggestion as the factor would only be released at the site of an injury where clotting would occur. The released PDGF would then stimulate only the cells immediately surrounding the injury. This is an essential mechanism for a hormone which has as its target connective tissue that is universally present throughout the body, thus no organ-specific factor could exist as with the liver or kidney. The stimulation of the vascular smooth muscle cells has suggested that the PDGF may also be involved in atherosclerosis [79–80]. An injury to a vessel wall, as in experimentally induced atherosclerosis, would trigger local clotting and release of PDGF which could then stimulate the surrounding vascular smooth muscle cells and begin the process of plaque formation.

The general conclusions seem to be that the responsive cell types in vitro and packaging of the PDGF within the platelet makes this factor an ideal subject for the growth factor involved in wound healing. This mechanism would obviously involve a different delivery system than during embryonic growth as only the cells in a limited area need respond.

Fibroblast growth factor. A protein similar to PDGF has been isolated and characterized from bovine brain and pituitary. This factor, fibroblast growth factor (FGF), is also a basic polypeptide with an isoelectric point of 9.6 and a molecular weight of 13,000 daltons [81, 82]. Chemically, this factor is strikingly similar to PDGF, but its biological activity clearly distinguishes the two. The FGF is a potent mitogen in vitro mesoderm-derived cells, especially vascular and corneal endothelial cells at the same level of purified PDGF, that is, 1 ng/ml or 10^{-10} M [83, 84]. Vascular endothelial cells grown in the presence of FGF form a tightly packed cell layer which is morphologically similar to that observed in vitro [85, 86]. The brain FGF has also been shown to be an angiogenic factor in vivo [87]. This may be related to its potent stimulation of the vascular endothelial cells mentioned above. It is interesting to speculate that the PDGF and FGF with their complementary activities for vascular tissues would work in concert to generate new vessels during wound repair. Purified FGF has been reported to stimulate the growth of bastemal cells of frogs [83, 88]. This has led to the suggestion that FGF, or a similar factor, may be involved in the initial stages of repair and regrowth of amputation injuries in lower vertebrates. This would certainly qualify it as a candidate for a wound hormone.

Kidney and liver growth factors. There is another instance where growth factor activity has been implicated in wound repair. This is the case of the regrowth of the function of certain organs after injury. This is commonly called compensatory growth and encompasses both the restoration of the original level of function and eventually the original size. There are two organs that we will discuss which are capable of compensatory growth, the kidney and the liver. The kidney is capable of returning to a significant portion of its original output following injury (either due to disease or experimentally by removal of

one of the pair) by both hypertrophy and hyperplasia [89]. Considerable evidence has accumulated to implicate humoral factors, renotropins, in this repair process. This has been done in several ways, either by injecting serum from uninephrectomized animals into test animals [90, 91] or in experiments with parabiotic pairs [92]. Biochemical evidence suggests that the renotropic factor may be a heat stable polypeptide of less than 25,000 daltons which is organ specific [93]. Renotropic factors have also been reported in urine [94, 95].

There is evidence that the regeneration of damaged liver tissue may also be under the control of humoral growth factors. A protein factor of 30,000 to 50,000 daltons has been identified in both the serum from partially hepatectomized rats and in the extracts of the regenerating liver [96–98]. This factor was found to be specific for liver in vivo with no activity on the kidney or spleen. A similar factor has been identified in the serum of rats with liver damage induced chemically by thioacetamide [99]. This factor was active in vivo upon injection into either rats or mice. These factors may be similar or identical to factors acting in normal developmental growth or even in neoplastic conditions as it has been shown recently that hepatic stimulatory factors are present in extracts of both weanling or regenerating adult liver [100] and Ehrlich ascites carcinoma fluid [101].

Neoplastic growth

The third biological situation in which growth factors play an important role is in the rapid cellular proliferation associated with the various neoplasms. Whether this abnormally high growth role is due to increased sensitivity to normal levels of growth factors or to increased production of new factors is under intense investigation.

Transforming growth factors. One of the most interesting aspects of growth factor research recently has been the discovery of a class of factors produced by tumor cells which have the ability, in vitro, to cause normal cells to morphologically resemble tumor cells. This discovery arose from the long observed phenomenon that tumor cells in culture required less serum to grow and survive than the corresponding normal cells. Todaro and Delarco [102] first described that certain sarcoma virus transformed cells bound far less labeled EGF than their normal counterparts or cells transformed by other means. These studies were expanded subsequently by the finding that the sarcoma virus-transformed cells produced a factor which bound to the EGF receptors on the cell surface [103, 104]. These sarcoma growth factors (SGF) were produced in such quantity that the available surface receptors were always occupied and thus the cells apparently possessed fewer receptors for labeled EGF.

The biological activity of the SGF is quite amazing when they are tested with cultures of normal cells. The SGF can induce in these normal cells all the biological manifestations of transformed cells normally seen in culture [103, 105]. This includes the stimulation of growth-arrested normal cells to continue division above a saturation level normally inhibitory to further growth and the ability of normal cells to form colonies in soft agar suspension culture. This property was formerly considered a prime difference between normal and transformed cells in culture. The morphology of the normal cells also changes to one resembling the transformed cells. The most interesting aspect of the biological effects of the SGF is that they are totally dependent on the continued presence of the SGF in the growth medium, and the normal phenotype is returned if the SGF-containing media is replaced with normal culture media. The EGF produces none of the effects previously mentioned.

The SGF from the culture media of murine sarcoma virus transformed fibroblasts while binding to the EGF receptors is distinct from the EGF biochemically and biologically. The SGF is a heat-stable, trypsin and thiol reagent sensitive polypeptide which does not cross-react with anti-EGF antibodies [103]. There are three active molecular weight species detectable by gel filtration: 25,000, 12,000, and 7,000. Polypeptides with the same biological properties have been extracted from the sarcoma virus-transformed cells grown in culture and from chemically induced tumors in mice [106] by an acid/ethanol extraction procedure. In this case the major biological activity was in the 7000-dalton species.

The discovery of the SGF and other transforming growth factors (TGF's) from animal and human tumors and tumor cell lines [104, 107] has resulted in the theory that tumor cells can maintain their rapid growth and anchorage independence in part by the autocrine production of their own growth factors [108, 109]. In many of their functions, the TGF's may resemble factors which are required during normal embryonic development. Recently, polypeptides which are indistinguishible both biochemically and in their biological activity have been isolated from mouse embryos [110] and from many non-neoplastic sources such as submaxillary gland, kidney, liver, heart and brain [111]. It has also been shown that during embryonic development, at certain stages there is more EGF-receptor binding activity than can be accounted for by immunoreactive EGF, also suggesting factors which bind EGF receptors but which chemically are different from EGF [112]. These findings suggested that the differences between neoplastic and non-neoplastic cells may be more quantitative rather than qualitative. The ongoing study of the TGF's will certainly lead to a better understanding of both the normal and abnormal growth control mechanisms.

Tumor angiogenic factors. Another growth factor found associated with neoplastic cells is the angiogenic stimulating activity. Angiogenesis, the formation of new blood vessels, is critical to the survival of solid tumors, and it is a long recognized phenomenon that tumor cells secrete angiogenesis factors, TAF's [113]. This has been observed by assaying both growing tumor fragments and various extracts in vivo using mainly in the chick chorioallantoic membrane or rabbit corneal micropocket assays. Recently, an in vitro assay using fetal bovine aortic endothelial cell cultures has been developed [114]. The angiogenic factor(s) must possess several biological activities, that is, it must be both chemotactic and mitogenic for vascular endothelial cells. The use of the in vitro mitogenic assay with endothelial cell cultures followed by the in vivo assays mentioned above has allowed the purification to a great degree of tumor angiogenic factor. The TAF was isolated originally as a large molecular weight complex containing RNA, protein, and carbohydrate [115] but subsequent investigations show that the biological activity resides in a very low molecular weight factor (less than 1000 daltons) whose structure still remains to be elucidated [114, 116, 117]. As with the transforming growth factors, similar angiogenic factors have also been identified from normal tissue sources [113, 118]. Two higher

molecular weight angiogenic factors, 50,000 and 70,000 daltons, have been isolated recently from bovine retina [119]. It did not appear that these high molecular weight factors were carriers for the low molecular weight factor similar to TAF as the activity was stable after acid treatment and denaturing reagents. The purification and final characterization of the TAF as well as those from normal tissues will be of great importance in the study of how tumors interact with the host animal. The survival importance of neovascularization to tumor growth is shown by the inhibition of tumor growth in vivo by infusion of a partially purified angiogenesis inhibitor [120]. This angiogenesis inhibitor was not directly inhibitory to the tumor cells in culture even at high concentrations.

Angiogenic factors present in nontumor tissue samples or serum are obviously involved in processes not involved with tumor growth. Angiogenesis is of importance in several other situations such as wound repair and in normal growth. As mentioned previously, FGF has been noted to possess angiogenic activity [87] possibly important for its potential role in wound healing and regeneration. It has also been noted that certain aspects of an inflammatory response also are capable of eliciting endothelial cell proliferation in vivo and neovascularization in vivo. For example, mitogen-activated spleen cells and the conditioned media from these cells is capable of inducing new vesicle in the chorioallantoic membrane assay of chicken eggs [121]. A macrophage-derived factor has been identified which can stimulate the proliferation of fibroblasts, vascular smooth muscle cells and vascular endothelium [122]. This factor was characterized as a nondialyzable, heat-labile, disulfide-containing protein. The migration of this factor through ion-exchange columns indicated that it was not PDGF or FGF.

The results linking inflammatory cells and vascularization are supported by observations resulting from thermal injury to rat skin [123]. In these studies, acute inflammation was initiated by a thermal injury of 60°C for 20 sec, which was followed by endothelial cell proliferation within 1 to 3 days and new vascularization at 5 to 7 days. Such effects could have been mediated by angiogenic factors released from cells participating in the inflammatory response.

Summary

In the preceding sections we have shown evidence that growth-promoting factors are involved in three basic situations. In normal embryonic development and function of mature organisms, growth factors such as NGF and EGF are of prime importance in supporting the necessary embryonic cell proliferation and the development of specific cell types. Other factors operate on subsets of mature cells during specialized functions such as inflammation. Included in this set would be factors such as CSF/MGF and Interleukin-2.

Another basic function of growth factors has been shown to be wound repair and organ regeneration. This includes the well characterized PDGF and FGF as well as the various renotropic factors and liver growth factors. As these factors must operate in mature organisms with many different cell types and similar cell types in many locations, more specificity is needed than in embryonic growth. This has resulted in the organ specific factors such as the renotropins and in the unique delivery system of the PDGF.

The recent discovery and characterization of the transforming growth factors has provided a possible connection between embryonic and normal developmental growth and the rapid cellular proliferation characteristic of tumor cells. The TGF not only interacts with receptors for normal growth factors such as EGF but are also detectable in low levels in normal tissue and embryos. The exact relationships between these various factors will have to await the determinations of more amino acid sequences for comparisons. The other tumor-related product, tumor angiogenesis factor, is also found in normal tissue and inflammatory reaction sites.

Any presentation of the biological function of the various growth factors must include their role in the growth control process. Obviously, growth factors are involved in the initiation at some point of division in sensitive cells. As shown here, this process can be regulated by limiting the cell types sensitive for certain factors or by limiting the distribution of the factor as with the PDGF. There is also evidence for the presence of inhibitors of cell growth with, in many instances, specificities analogous to the growth factors presented here. We will not present a detailed discussion of the various growth inhibitors and their possible interactions with growth stimulators. A major obstacle to the assignment of roles to growth inhibitors has been a lack of well defined inhibitors with which to study their biological interactions. To date, no inhibitor has been characterized to the extent that EGF or NGF has been. This, combined with the inherent difficulties in studying growth inhibition free of nonspecific toxic effects greatly hindered the development of theories concerning interactions of growth factors and inhibitors.

Generally, there are reports of growth inhibitors for most of the stimulators mentioned here. For example, there is considerable evidence for epidermal cell inhibitors [124, 125], as well as smooth muscle [126], liver [127–129], granulocyte [130, 131], lymphocyte inhibitors [132], and many others [6]. It has been proposed that cell growth is controlled by cell-specific, endogenous reversible inhibitors of cell division usually termed chalones [6, 7]. These chalones function normally to restrain cells from proliferation, possibly by preventing the actions of growth factors. There is some experimental evidence to suggest that the action of inhibitors may be counteracted by specific growth factors [133, 134].

The general function of growth factors and growth inhibitors (chalones) is to keep the cell number in balance such as to maintain tissue homeostasis. The final determination of the relative role each plays in this process is yet to be resolved. There are many functions of a mature organism which require rapid local proliferation of a small population of cells or the continued proliferation of one specific cell type. Both growth stimulators and inhibitors are involved in the initiation, maintenance, and final cessation of cellular proliferation.

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